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ORIGINAL PAPER

Dual-chambered bio-batteries using immobilized mediator electrodes

A. B. Hoffman · S. Suresh · R. W. Evitts · G. F. Kennell · J. M. Godwin

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Abstract Methylene blue was immobilized on 304L stainless steel to investigate a potential cost-effective, durable, and high performance composite electrode for use with microbial applications, such as bio-batteries and microbial fuel cells. The composite electrodes were tested in dual-chamber bio-batteries with pure cultures of *Escherichia coli* K-12 or *Shewanella oneidensis* MR-1 and the results were compared to those obtained using bare graphite electrodes. The maximum power generated using the composite electrodes was 39.35 mW m^{-2} in bio-batteries using *E. coli* K-12, and 60.05 mW m^{-2} in bio-batteries using *S. oneidensis* MR-1. Compared to graphite electrodes, the bio-batteries using composite electrodes showed a 6- and 2.5-fold increase in the maximum power density, using pure cultures of *E. coli* K-12 and *S. oneidensis* MR-1, respectively. The composite electrodes did not inhibit bacterial growth in the bio-batteries and were shown to improve performance (both in terms of power output and current density) over conventional graphite electrodes.

Keywords Bio-battery · Immobilized mediator electrodes · Stainless steel electrodes · Polarization · Microbial

1 Introduction

Microbial fuel cells (MFC) have been proposed as an alternative source of energy [1]. However, multiple aspects of MFC technology must still be improved before they can become viable for energy production. One of the primary aspects to be considered is the optimization of the electrodes such that they are cost effective, durable, and efficient. Previously, electrodes such as platinum, gold, graphite, glassy carbon, and stainless steel were investigated for use in MFC. While noble metals such as platinum and gold have high performance characteristics for electron transfer they are relatively expensive. Zhang et al. [2] compared graphite felt, carbon paper, and stainless steel mesh as bio-cathodes in a single chamber MFC and determined that graphite felt performed better. Though graphite and glassy carbon are not excessively expensive, they are brittle and thus impractical for large-scale industrial use. Stainless steel has also been suggested as a solution to the expensive or impractical alternatives; however, stainless steel has only been researched and shown to perform in sea floor MFC where the cell is driven by the potential difference between the aerobic and anaerobic environments present at the sea surface and sea floor, respectively [3]. The seawater MFC do not exhibit high performance and are not fully understood due to the mixed microbial cultures present in the sea environment [3]. Stainless steel has also been used as the basis for an air cathode in a MFC with an applied polymer diffusion layer and catalyst [4]. Also, stainless steel meshes were flame treated to form carbon nanostructures which improved the power density by 60-fold compared to plain stainless steel mesh anodes [5]. More recently, stainless steel has been used as the basis for a bio-anode with a redox layer to facilitate electron transfer [6].

One proposed solution for a cost-effective, durable, and high performance electrode feasible for MFC is the use of immobilized electron mediators [7]. Electron mediators assist electron transfer from the microbial solution to the

A. B. Hoffman · S. Suresh · R. W. Evitts · G. F. Kennell (✉) · J. M. Godwin
Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon, SK S7N5A9, Canada
e-mail: glyn.kennell@usask.ca

electrode, thus increasing the performance of MFC. Using immobilized electron mediators is beneficial because the mediators do not need to be separated from the solution after use, as is the case with soluble electron mediators. Electron mediators may be immobilized onto an inexpensive and strong material, such as stainless steel, which makes for a cost-effective, durable, and high performance electrode that is useful for bio-batteries and MFC.

In previous research, various approaches have been used to immobilize electron mediators. Park and Zeikus [8] used transition metal ions entrapped in carbon electrodes to achieve a 1,000-fold increase in the amount of electrical energy produced by MFC, in comparison with fuel cells which did not have entrapped metal ions in the electrodes. Feng et al. [9] examined conductive polymers doped with anionic redox compounds on a carbon felt electrode and achieved a 13-fold increase in the maximum power density obtained from the MFC, as compared to a microbial fuel cell using unmodified carbon felt. Prieto-Simón and Fàbregas [10] researched the variations between mediators in solution, mediators entrapped in epoxy-composites, and mediators absorbed or polymerized on the electrode surface for use in dehydrogenase-based biosensors. They concluded that mediators polymerized on the electrode surface were the most suitable for this application due to the reproducibility and repeatability of the results, while still maintaining a suitable level of sensitivity in the biosensor.

In this research, immobilizing an electron mediator on 304L stainless steel was performed by applying a layer of polypyrrole to the polished stainless steel followed by electro-polymerizing the redox dye (methylene blue) on top of the polypyrrole surface. This polypyrrole- and methylene blue-coated stainless steel electrode was then tested in bio-batteries containing either *Escherichia coli* K-12 or *Shewanella oneidensis* MR-1. The research here was performed using bio-batteries rather than MFC; however, the electrochemical performance of the microbe/electrode combination should be similar in either physical setup. The sole distinction between MFC and bio-batteries is that MFC have the substrate oxidized at the anode replenished, either continuously or intermittently, whereas this is not the case for bio-batteries [11].

Previous research has been conducted using *E. coli* and *S. oneidensis* in MFC; however, the past research differs from this study in the type of electrodes used. MFC using pure cultures of *E. coli* have been studied with bound electron mediators on graphite, such as a Mn^{4+}-graphite electrode and a neutral red covalently linked woven graphite electrode [8], with graphite and methylene blue mediator in solution [12], and with carbon paper and neutral red mediator in solution [13]. MFC using pure cultures of *S. oneidensis* have been studied primarily using graphite felt electrodes, with and without the soluble electron mediator anthraquinone-2,6-disulfonate [14]. The work described here represents a study in which the performance of bio-batteries with *E. coli* K-12 or *S. oneidensis* MR-1 using immobilized mediator electrodes were analyzed and compared to microbial fuel cell performances using conventional graphite electrodes.

2 Materials and methods

2.1 Microbial cultures and medium

The *E. coli* K-12 used in this study was a pure culture from the American type culture collection. It was used as a model organism for bacteria that make use of external mediators to transfer electrons. The *S. oneidensis* MR-1 was a pure culture obtained from the Belgian co-ordinated collections of microorganisms (BCCM/LMG), and was chosen as a model organism for bacteria that do not require external mediators to facilitate electron transfer. This electron transfer is more facile due to electrically conductive pilus-like appendages located on *S. oneidensis* and affects the overall cell voltage. Starter cultures of both bacteria were grown in a LB-Miller medium at an ambient room temperature of 22 °C.

2.2 Electrochemical equipment

Gamry™ Instruments were used for all electrochemical polymerizations and experimental measurements. A Gamry™ Reference 600 Potentiostat, along with the Gamry PHE200™ Physical Electrochemistry Software was used for polymerizations as well as for cyclic voltammetry potential sweeps to obtain polarization curves. A Gamry™ ECM8 Multiplexer was used in conjunction with the Gamry Reference 600 Potentiostat to obtain the open circuit potentials of the MFC.

2.3 Electrode coating preparation

Stainless steel 304L rods obtained from Steelmet (Saskatoon, Canada) were cut to appropriate sizes and sanded with 600 grit sandpaper, followed by polishing with 0.3 μm alumina to give a mirrored finish. The polished rods were rinsed with ethanol to remove any metal particles and excess water that may otherwise have interfered with polymerization. A polished steel rod, along with a graphite counter electrode and saturated calomel reference electrode were immersed in a solution of 0.35 mol L^{-1} pyrrole, 0.08 mol L^{-1} sodium salicylate, and a small amount of phosphoric acid to reduce the pH to 4.5. Polymerization of pyrrole onto the stainless steel rod was done galvanostatically with a current density of

6 mA cm^{-2}, for a time of 180 s. The polypyrrole layer can be visually confirmed by the black coating on the stainless steel rod. The polypyrrole-coated rod, along with a graphite counter electrode and saturated calomel electrode, was immersed in a solution of 0.001 mol L^{-1} methylene blue, 0.1 mol L^{-1} potassium nitrate, 0.025 mol L^{-1} sodium borate, and just enough potassium hydroxide to adjust the solution to the desired pH of 9.5. Methylene blue was polymerized onto the polypyrrole-coated steel rod by running cyclic voltammetry at a rate of 100 mV s^{-1} for 8 cycles, ranging from a minimum potential of −0.5 V_{SCE} to a maximum potential of 1.05 V_{SCE}. The polypyrrole/polymethylene blue coating appears purplish in color. The coated electrodes were stored in reverse osmosis water until use.

2.4 Experimental set-up

Experiments were conducted in dual-chamber bio-batteries operated in batch mode. Each chamber held 400 mL of solution and were connected via an H-bridge, with a CMI-7000 cation exchange membrane (supplied by Membranes International Inc., USA) separating the solutions. The cathode chamber of the bio-battery contained an autoclaved solution of 0.05 mol L^{-1} potassium ferricyanide and 0.1 mol L^{-1} phosphate buffer with a pH of 6.3. A sterile graphite rod counter electrode and saturated calomel electrode were placed in the cathode chamber, which was then sealed. The anode chamber contained a solution which was an autoclaved LB-Miller medium with additional 10 g L^{-1} glucose for the *E. coli* K-12 experiments or 0.02 mol L^{-1} sodium-L-lactate for the *S. oneidensis* experiments. Bacteria were grown aerobically for no less than 3 days before being centrifuged (at 5000 RCF for 30 min for *E. coli* K-12 and at 3000 RCF for 30 min for *S. oneidensis* MR-1) and subsequently used to inoculate the anode chamber. An electrode, either graphite or composite, was placed in the anode chamber. The anode chamber was sealed and purged with 100 mL min^{-1} of filtered nitrogen for 1 h, after which a continuous 25 mL min^{-1} of filtered nitrogen was passed through each of the anode chambers to assure anaerobic conditions were maintained. Magnetic stirrers were used throughout the experiment in both anode and cathode chambers to induce mixing, which prevented settling and ensured a uniform composition within each chamber.

2.5 Analysis of electrode performance

The bio-batteries were analyzed by measuring and plotting the open circuit potentials and polarization curves, as well as investigating and recording the biomass concentrations in the anode chamber and internal resistances of the bio-batteries. The open circuit potentials were determined after the bacteria growth in the anode had reached steady state. Polarization curves were obtained by running a cyclic voltammetry scan at a rate of 0.15 mV s^{-1} at a potential running from the measured open circuit potential to the short circuit potential of the anode chamber with respect to the cathode chamber. The final current values were divided by the immersed (geometric) surface area of the anode electrode to give current density. Biomass concentrations were measured after completion of the electrochemical measurements by centrifuging 300 ml of *E. coli* K-12 anode solution and the full solution for experiments with *S. oneidensis* MR-1 at 5000 RCF for 30 min, followed by drying at room temperature for 5 days, at which point the weight of the dried cells was measured.

3 Results and discussion

Bio-batteries were constructed and tested using the methods described in Sect. 2 of this article. The bio-batteries and electrodes were then analyzed to better understand the role of the composite immobilized mediator electrode in MFC and bio-batteries.

3.1 Immobilization of mediator

Figure 1 shows SEM images of bare polypyrrole (a) and polypyrrole/methylene blue (b)-coated stainless steel electrodes. Polypyrrole is the first layer electrodeposited onto the stainless steel. Subsequently, methylene blue is applied on top of this polypyrrole coating, as described in Sect. 2 of this article. From the figure, it is clear that the polypyrrole/methylene blue or the second layer of coating is more uniform than the bare polypyrrole coating. It is also clear from the figure that both polypyrrole and methylene blue have formed an adhesive coating on the stainless steel electrode. This immobilized mediator facilitates electron transfer at its surface. Figure 2 shows the schematic representation of electron transfer from the cells, to the stainless steel electrode, with the aid of immobilized mediator.

3.2 Open circuit potentials

Open circuit potential measurements can help to indicate the relative efficiency with which the microbes convert substrate to energy [15]. The open circuit potentials were taken as the final, stable readings obtained after the bacteria growth in the anode chamber had reached steady state. The average open circuit potential of the bio-batteries with *E. coli* K-12 (based upon three trials) was 0.392 V using the composite electrodes and 0.471 V using graphite

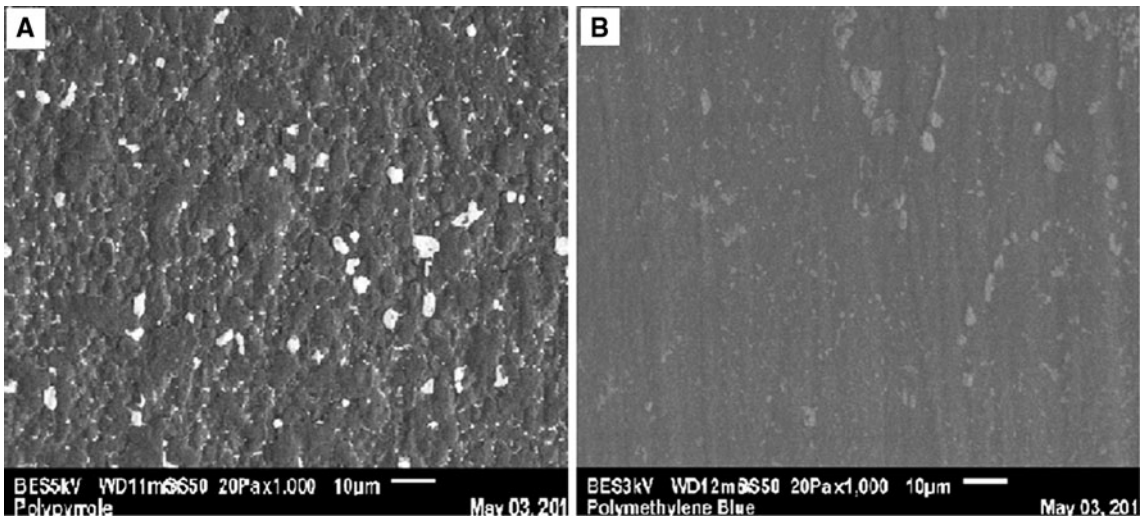

Fig. 1 SEM images of **a** bare polypyrrole-coated SS electrode and **b** polypyrrole- and methylene blue-coated SS electrode

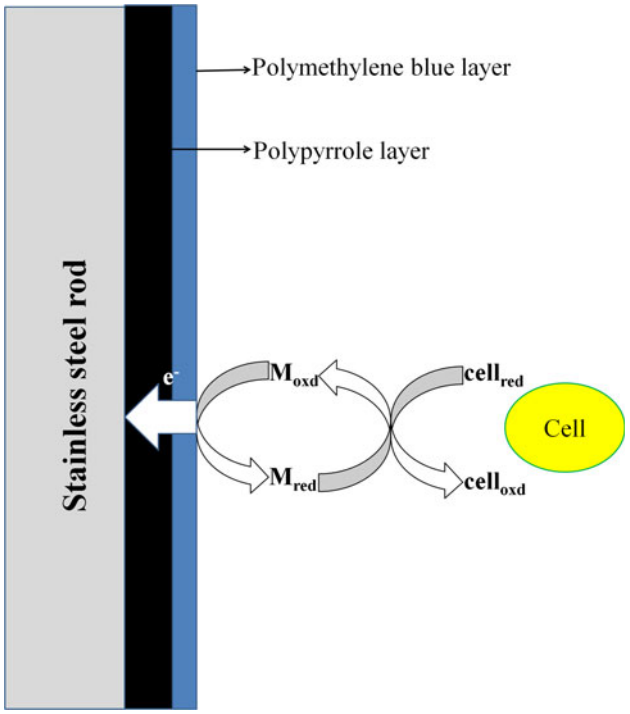

Fig. 2 Schematic representation of electron transfer

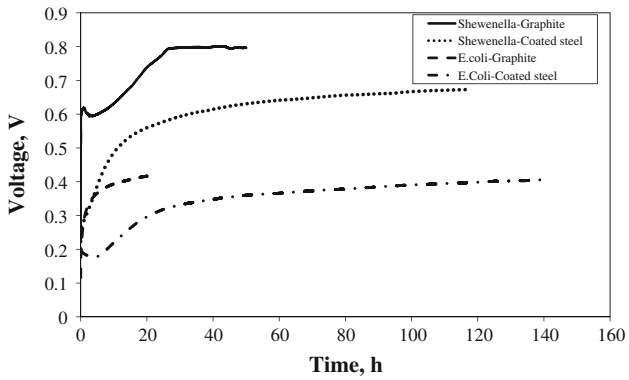

Fig. 3 Open circuit potentials for *E. coli* K-12 and *S. oneidensis* MR-1 experiments using coated steel and graphite electrodes

electrodes. Slight variance in pH differences between the anode and cathode caused between 0.0047 and 0.0177 V of discrepancy in the voltage values obtained. A plot of the open circuit potential for the experiment using the composite and graphite electrodes with *E.* coli K-12 and *S. oneidensis* can be seen in Fig. 3. The open circuit potential measurement was started when purging of the anode chamber was begun; therefore, at early time periods (up to ~10 min) the figure shows the transition between aerobic and anaerobic growth of the *E. coli* K-12.

The average open circuit potential of the bio-batteries with *S. oneidensis* MR-1 were 0.668 V using the composite electrodes and 0.779 V using graphite electrodes. Slight variance in pH differences between the anode and cathode caused between 0.0124 and 0.0148 V of discrepancy in the voltage values obtained. From the open circuit potential plots, it can be seen that the experiment using the composite electrode took significantly longer to reach a final, stable open circuit potential than the experiment using a graphite electrode.

For the trials described above, using bacteria, the average open circuit potentials obtained for the graphite electrodes were higher than for the coated steel electrodes. This indicates the possibility for reduced surface overpotentials and increased battery efficiencies, due to an increased efficiency in the electron transfer occurring at the surface of the composite electrode. The important comparison parameter, however, between the different types of electrodes is the current obtained at various potentials. To

determine the differences in currents obtained for the different types of electrodes, cyclic voltammetry was used to produce polarization curves. These results are presented in the subsequent section.

3.3 Polarization curves

Polarization curves were plotted using a slow (0.15 mV s^{-1}) forward potential sweep and dividing the obtained current value by the geometric surface area of the immersed anode electrode. The performance potential of bio-batteries can be expressed using polarization curves, as they show information regarding the open circuit voltage of the bio-battery set-up, the maximum current obtained, and also the characteristics of the voltage–current behavior, which gives an insight into the dominant internal resistances [15].

Representative cyclic voltammetry scans for experiments using the coated steel electrode, both with and without *E. coli* K-12, are shown in Fig. 4. The slow potential sweep of 0.15 mV s^{-1} allows the scan to approach steady state at each point, giving a good representation of the voltage–current behavior of the system. Figure 4 shows the increase in current generation due to the presence of bacteria. For the cyclic voltammetry scan with *E. coli* K-12 there is a slight separation of the forward and reverse curves due to a loss of stored charge, whereas with the cyclic voltammetry scan without *E. coli* K-12 there is a larger separation, where the majority of the current can be accredited to stored charge in the anode. Polarization curves for the experiments using *E. coli* K-12 are shown in Fig. 5, and those for the experiments using *S. oneidensis* MR-1 are shown in Fig. 6. The average short circuit (maximum) current density values obtained with *E. coli* K-12 were 316.7 mA m^{-2} with the composite electrode and 51.2 mA m^{-2} with a graphite electrode. For the experiments with *S. oneidensis* MR-1, the average short circuit current density values obtained were 302.4 mA m^{-2} with the composite electrode and 77.7 mA m^{-2} with a graphite electrode. These results show an increase in the short circuit current density by a factor of 6.2 for the experiments using *E. coli* K-12 and by a factor of 3.9 for the experiments using *S. oneidensis* MR-1, when using coated steel electrodes as opposed to graphite electrodes. As current is an important comparison parameter between the different types of electrodes and is indicative of the ease in which the electrons are transferred within the system, it is apparent that the coated steel electrodes are more efficient at transferring electrons than the graphite electrodes.

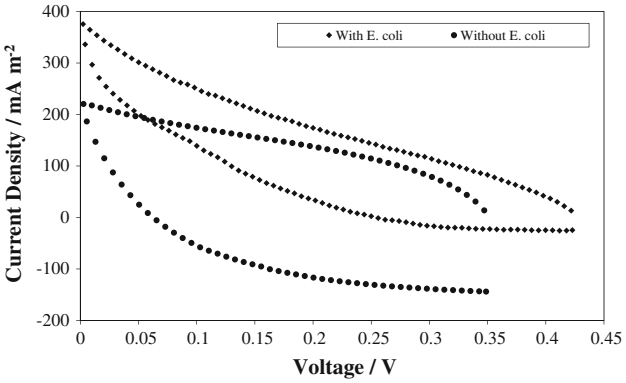

Fig. 4 Representative cyclic voltammetry scans for experiments using coated steel electrode, both with and without *E. coli* K-12

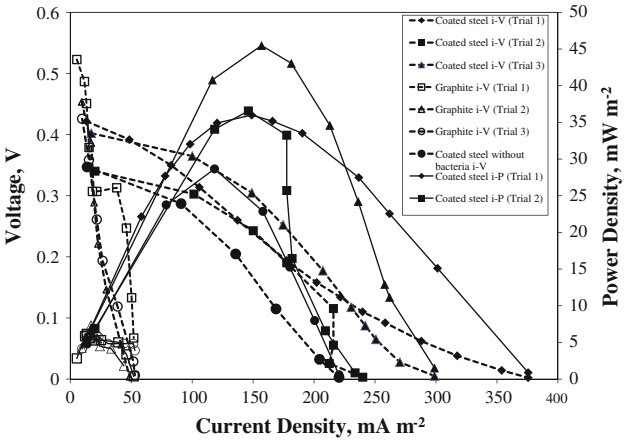

Fig. 5 Polarization (i–V) and power density (i–P) *curves* for *E. coli* K-12 experiments

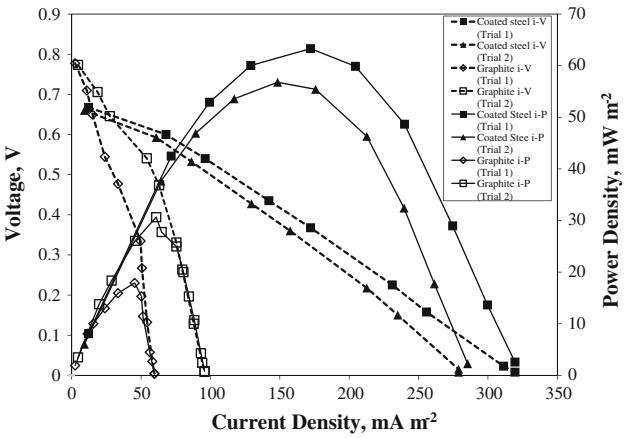

Fig. 6 Polarization (i–V) and power density (i–P) *curves* for *S. oneidensis* MR-1 experiments

3.4 Power density curves

Power density curves for experiments with *E. coli* K-12 are shown in Fig. 5, and for experiments with *S. oneidensis* MR-1 in Fig. 6. The power density curves show the maximum power density obtained from the bio-batteries and the corresponding current density produced. The average maximum power density in the bio-batteries using *E. coli* K-12 was 39.35 mW m^{-2} for experiments using the coated steel electrode, and 6.49 mW m^{-2} for experiments using a graphite electrode. The average maximum power density in the bio-batteries using *S. oneidensis* MR-1 was 60.05 mW m^{-2} for experiments using the coated steel electrode, and 24.28 mW m^{-2} for experiments using a graphite electrode. These results show a 6-fold increase in the maximum power density obtained using coated steel electrodes as opposed to graphite electrodes for experiments using *E. coli* K-12, whereas a 2.5-fold increase was observed similarly for experiments using *S. oneidensis* MR-1. From these findings, it is clear that the use of immobilized mediator electrodes, as opposed to conventional graphite electrodes, provide a greater power production from both types of bacteria. In the case of *S. oneidensis* MR-1, the improvement in power production (when using immobilized mediator electrodes as opposed to conventional graphite electrodes) is less due to electrically conductive nanowires produced by the bacteria. According to Gorby et al. [17], the nanowires produced aid in transferring electrons from the solution to the electrodes, making it unnecessary to use mediators in *S. oneidensis* MR-1-driven MFC. The power density obtained using bare graphite electrodes is, therefore, greater in the presence of these bacterial nanowires (such as in experiments using *S. oneidensis* MR-1) than in the presence of *E. coli* K-12, which generally requires redox mediators to be used [17].

3.5 Comparison with literature values

Comparisons of the open circuit voltage, maximum current density, and maximum power density with literature values can be found in Table 1. For the experiments with *E. coli* K-12, the open circuit potential obtained from literature is comparable to the open circuit potential obtained in this study for the experiments using graphite electrodes, with slight differences in that the literature reference used soluble methylene blue in the anode and hexacyanoferrate in the cathode. The maximum power density obtained from the literature source is much larger than either value obtained in this study, which can be attributed to the significant amount (0.012 mol L^{-1}) of soluble mediator present in the anode, as well as the hexacyanoferrate used to improve the cathode performance [12].

For the experiments with *S. oneidensis* MR-1, the open circuit potential obtained from literature [16] is comparable to the open circuit potential obtained in this study for the experiments using graphite electrodes. The maximum power density and maximum current density obtained from literature (for a mediatorless miniature microbial fuel cell with a graphite felt electrode) [16] were less than those obtained using either graphite or coated steel electrodes in this study. The plain graphite electrode showed a 3-fold increase in the maximum power and maximum current densities as compared to the literature values, whereas the coated steel electrode showed an increase in maximum power density by a factor of 7.3 and an increase in maximum current density by a factor of 12.5, compared to the literature values.

For each bacteria type, similar open circuit voltages obtained between experiments using different types of electrodes indicate that the bacteria are metabolizing in a similar way. The differences in current densities obtained indicate the relative ease in which electrons are transferred within the system; higher current densities suggest relatively easier electron transfer between the solution and electrode. Higher power densities suggest potentially higher power production and are, therefore, evidences of superior bio-battery performance.

3.6 Resistances of bio-battery setup

The average ohmic resistances of the bio-battery setups with *E. coli* K-12 and *S. oneidensis* MR-1 were 77.0 and 72.4 Ω, respectively. The ohmic resistance consisted of the resistances of ion conduction through the anode chamber and cathode chamber solutions as well as ion conduction through the cation exchange membrane. The overall internal resistance is comprised of the ohmic resistance as well as the resistance within the microbial metabolism and reaction with the mediator (in the case of the coated electrodes) and the charge transfer resistance at the surface of the electrode. The overall internal resistance, calculated from the slope of the polarization curve, indicated that the ohmic resistance accounted for roughly 5.16 % of the overall internal resistance using the coated steel electrodes and 0.63 % of the overall internal resistance using graphite electrodes for the *E. coli* K-12 experiments. For the *S. oneidensis* MR-1 experiments, the ohmic resistance accounted for roughly 2.41 % of the overall internal resistance using the coated steel electrodes and 0.54 % of the overall internal resistance using graphite electrodes. The reason for the relatively higher portion of the internal resistance being due to the ohmic resistance is that the immobilized mediator electrodes decrease the resistance to electron transfer at the electrode surface. As ohmic resistances remain relatively constant, they subsequently

Table 1 Comparison of electrode performance in this study with literature

Comparison parameters	*Escherichia coli* K-12			*Shewanella oneidensis* MR-1		
	Present (coated steel)	Present (graphite)	(Graphite with soluble methylene blue) [12]	Present (coated steel)	Present (graphite)	(Graphite felt, mediatorless miniature microbial fuel cell) [16]
Open circuit potential (volts)	0.392	0.471	0.462[a]	0.668	0.779	0.794[b]
Maximum current density (mA m^{-2})	323.19	52.84	–	302.40	77.69	24.26[b]
Maximum power density (mW m^{-2})	39.35	6.49	151.6[a]	60.05	24.28	8.2

[a] Not directly comparable due to hexacyanoferrate being used in the cathode, hexacyanoferrate is known to improve the cathode performance considerably [12]

[b] Taken from graph values

represent a larger percentage of the overall resistance. The immobilized mediator electrodes were shown to reduce the overall internal resistance by a factor of 8.3 for the experiments with *E. coli* K-12 and by a factor of 4.5 for the experiments with *S. oneidensis* MR-1. Therefore, it has been demonstrated that the coated steel electrode facilitated electron transfer for the cases examined.

3.7 Biomass concentration

Biomass concentrations were calculated by weighing a centrifuged and dried portion of the anode solution after completion of each experiment. The average biomass concentration, upon completion of the experiments, for the *E. coli* K-12 anode solution using the coated steel electrode was 0.0748 g in 100 mL, and using the graphite electrode was 0.0846 g in 100 mL. The average biomass concentration upon completion of the experiments for the *S. oneidensis* MR-1 anode solution using the coated steel electrode was 0.0536 g in 100 mL, and using the graphite electrode was 0.0437 g in 100 mL. The discrepancies in biomass concentration values between coated steel electrodes and graphite electrodes in *E. coli* K-12 and *S. oneidensis* MR-1 experiments is likely due to experimental variance, and is evidence that the coated steel electrode did not inhibit the growth of either bacteria.

4 Conclusions

The performance of a bio-battery using methylene blue-immobilized mediator on a stainless steel electrode showed improvement over the performance of a plain graphite electrode when used with pure cultures of either *E. coli* K-12 or *S. oneidensis* MR-1. When used in a bio-battery with *E. coli* K-12, there was a 6-fold increase in both the maximum current density and the maximum power density obtained when using an immobilized mediator electrode as opposed to a plain graphite electrode. When used in a bio-battery with *S. oneidensis* MR-1, the maximum current density obtained increased by a factor of 3.9 and the maximum power density obtained increased by a factor of 2.5 when using an immobilized mediator electrode as opposed to a plain graphite electrode.

The immobilized mediator electrodes provided higher current and power densities than plain graphite electrodes without inhibiting the growth of the *E. coli* K-12 or *S. oneidensis* MR-1 bacteria. These immobilized mediator electrodes could, therefore, have the potential to be used as cost-effective, durable, and high performance electrodes in both bio-batteries and MFC.

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